Morphological Studies on Entomogenous Stem Galls of *Microgramma squamulosa* (Kauf.) Sota (Polypodiaceae)

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The cells, tissues and organs of many plants are subject to the effects of a series of factors which may result in pathological growth, characterized by disease and loss of function or atypical growth, which involves changes in normal patterns of development and differentiation (Bloch, 1965; Rohfritsch & Shorthouse, 1982). One of the more complex forms of atypical growth is that known as gall. Galls are structures which originate through processes of hypertrophy and hyperplasia, inhibition of growth or cytohistological modification, in response to the attacks of gall-inducing organisms (Mani, 1964).

More than 15,000 types of gall are at present known; they are found on algae, fungi, lichens, bryophytes, pteridophytes, gymnosperms and angiosperms (Mani, 1964). Gall inducers are also widely varied, and include algae, lichens, fungi, bacteria, viruses, nematodes, arachnids and insects. The principal cause of gall formation is insects, and of the groups known to participate in this phenomenon, the most important in terms of frequency and importance are Coleoptera, Hemiptera, Diptera and Lepidoptera (Mani, 1964; Occhioni, 1979; Fernandes & Martins, 1985).

Fossil evidence of various levels of interaction between pteridophytes and the arthropods dates back to the Devonian period, and in some cases suggests complex associations (Scott *et al.*, 1985). Even so, entomogenous galls are not frequently found among cryptogams (Houard, 1933; Mani, 1964; Fernandes, 1987). Interactions between insects and pteridophytes have been studied by Mohan-Daniel & Chandrasekar (1986). According to Fernandes (1987), of the principal types of gall-inducing agents only insects are important for pteridophytes. Mani (1964) claims that the family Polypodiaceae, comparatively young and rich in number of species, is the best represented in terms of numbers of galls. The same author points out that none of the galls which occur in this family are known to be caused by Lepidoptera.

This work is concerned with some morphological aspects of entomogenous galls found on the stems of *Microgramma squamulosa,* an epiphytic Brazilian fern, and with the understanding of the inter-relationship between plant and inducing agent.

MATERIALS AND METHODS

The plant material under investigation is the epiphytic fern *Microgramma squamulosa* (Kaulf.) Sota (Polypodiaceae), which grows on trees of *Tipuana tipu* Benth. (Fabaceae), used as ornamentals in streets in the suburb of Bela Vista, City of São Paulo, Brazil. Aerial stems, both with and without galls, were collected and packed in damp closed plastic bags for purposes of transport. Analysis of the external morphology was based on

observation of the form of the galls, of their size at various stages of development, and of their position on the plant.

Stem segments with galls and unaffected segments were both used for anatomical studies. The samples were cut in pieces, put into Petri dishes containing damp filter-paper, and then transferred to FAA (formalin: acetic acid: ethyl alcohol 50° GL, 1:1:18, v/v) in accordance with Johansen (1940). After fixing, samples for study by light microscopy were dehydrated in an ethyl alcohol series and embedded in paraffin wax, as described by Johansen (1940) and Sass (1951). Serial transverse and longitudinal sections were then obtained and stained with Safranin and Fast Green (Moore, 1936). Some of the samples, after fixing, were dehydrated in a ketone series, and embedded in glycol-metacrylate (Reichert-Jung Historesin) as described by Beryln & Miksche (1976). Transverse and longitudinal sections were stained with Toluidine Blue (O'Brien *et al.*, 1964). Samples for electron microscopy were obtained after fixing, dehydrated in a ketone series, dried at critical point with CO_2, and prepared with gold and palladium as described by O'Brien & McCully (1981). Observation was carried out at 15–20 Kv. The most important aspects of morphology were recorded through photomicrographs and electromicrographs. Drawings were made with the help of a camera lucida attached to a stereomicroscope.

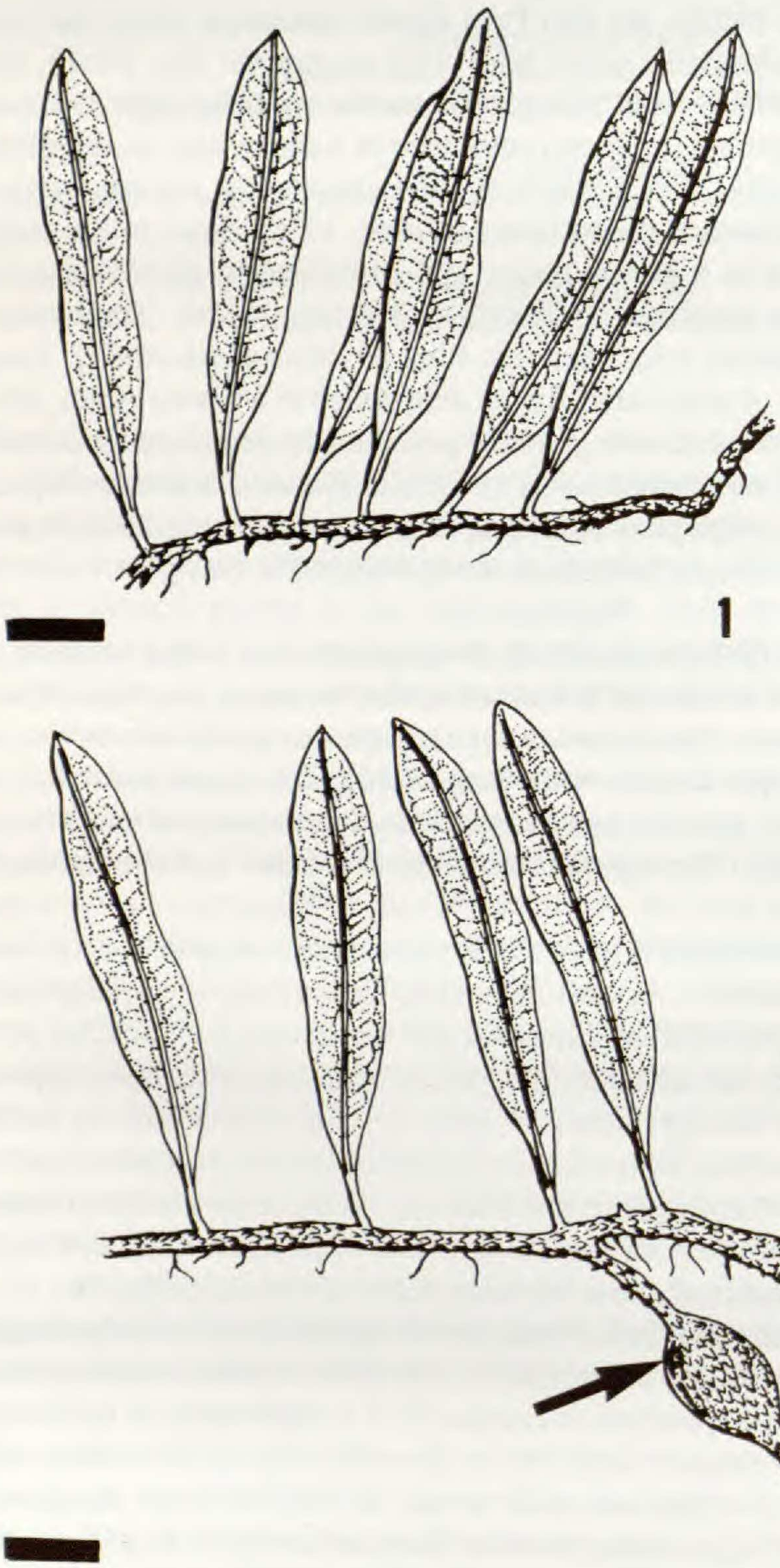
Results

Microgramma squamulosa (Polypodiaceae) is an ephiphytic fern with a creeping stem (Fig. 1), densely covered with scales; the leaves are spread in such a way that nodes and internodes can be distinguished. The leaves occur only on the dorsal side of the stem, while the adventitious roots appear only on the ventral side. This organization has some effect on the internal structure; the stele is dorsiventral in its structure, although in cross-section the stem is still circular. The organization of the leaf veins is characteristic (Fig. 2).

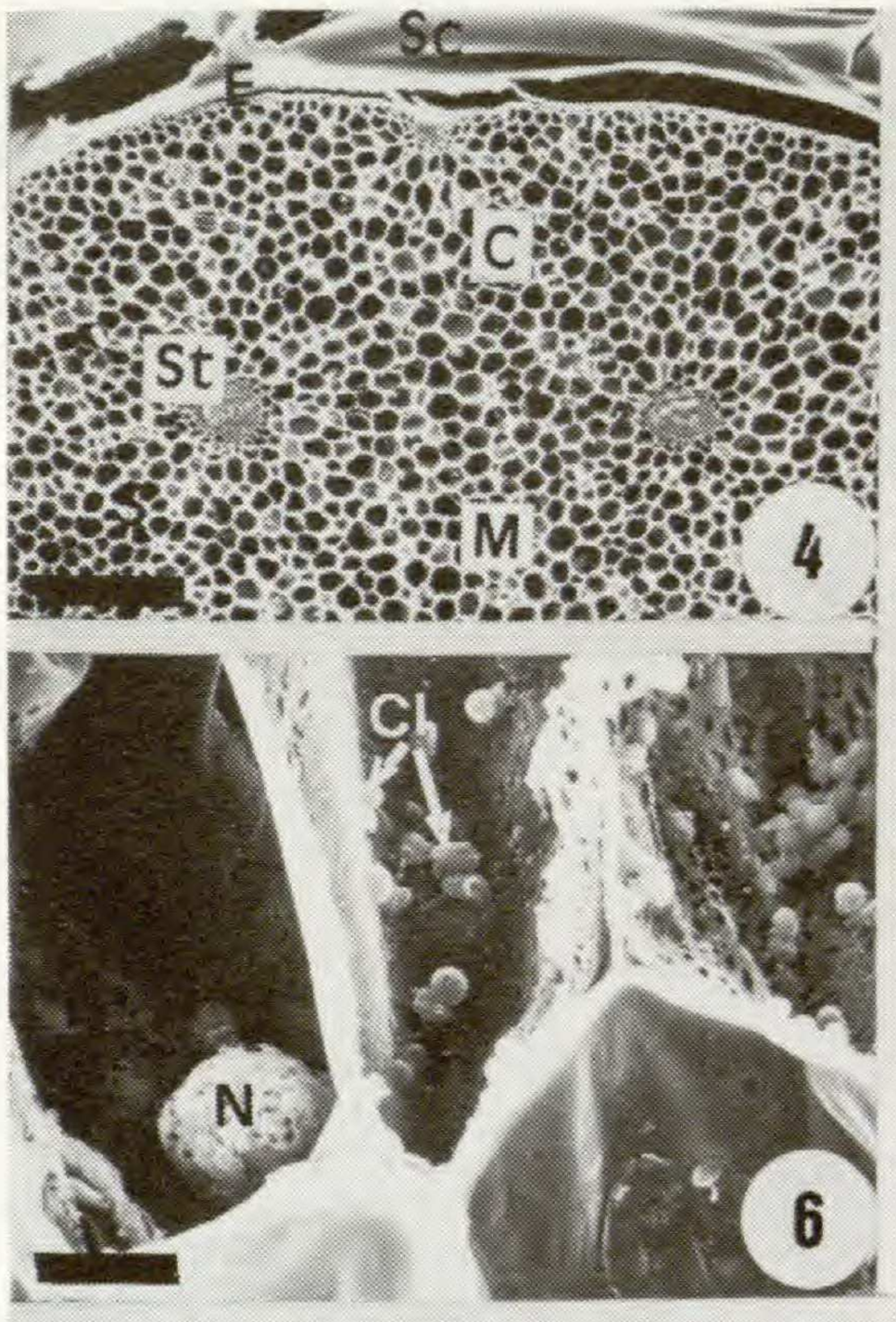
Stems of *M. squamulosa* with galls (Fig. 3) display peculiar characteristics. The affected region is fusiform in appearance. At least in external appearance, it no longer has the dordiventral arrangement of the unaffected regions. The dense covering of scales persists in the stem, but the leaves and the adventitious roots are generally lost. There appears to be no preferential region for the appearance of galls, they are found both on the main stem axis and on the branches, and occur on recent branches and on older stem regions.

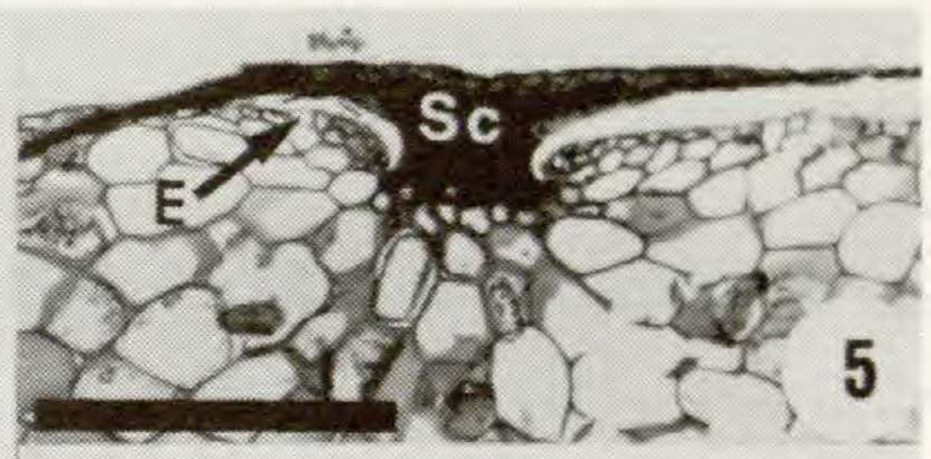
The gall inducer is a moth (Lepidoptera, Gelechiidae). At the more advanced stages of gall development a small orifice may be seen close to one of its extremities; this is previously prepared by the larva, and from it the inducing insect will emerge (Fig. 3).

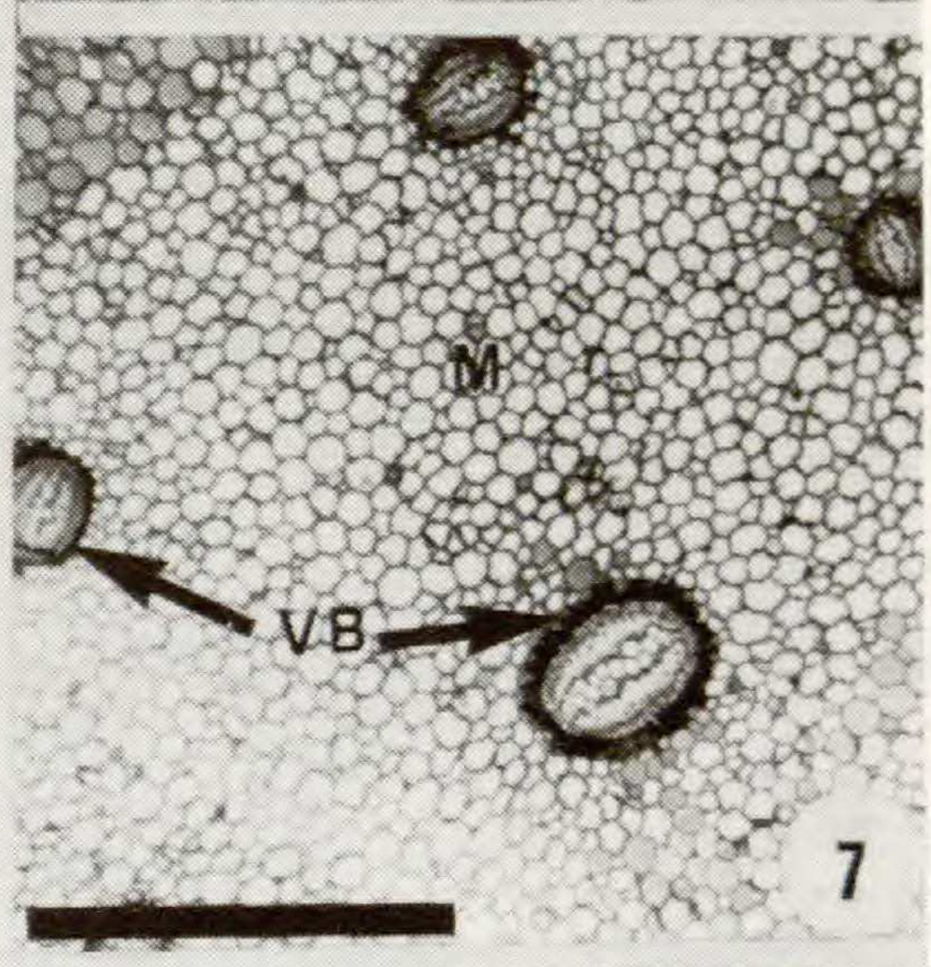
The internal structure of the unaffected stem on *M. squamulosa* is fairly simple. A cross-section shows, from the outside in, epidermis with scales, cortex, central cylinder or stele, and medulla (Fig. 4). The epidermis is composed of a single layer of quadrangular cells, and is covered by a fine cuticular layer and many scales (Fig. 5). The cortex and the medulla are made up basically of parenchymatic tissue, the cells of which are photosynthetic (Fig. 6). The stele of *M. squamulosa* is of the dictyostelic type – a cylindrical stele interrupted by numerous foliar lacunae (Fig. 7) –, so as to separate it into five or more meristeles. Each meristele (Fig. 8) is surrounded by the schlerenchyma sheath and the endodermis. The sclerenchyma (Fig. 9), or supporting tissue, is reduced; it is limited to a layer of quadrangular cells which surround the vascular bundles as a protective sheath. The cells display a brown-colored reinforcement in the tangential and radial walls. The endodermis (Fig. 9) may be distinguished by the regular cell arrangement; the cells are

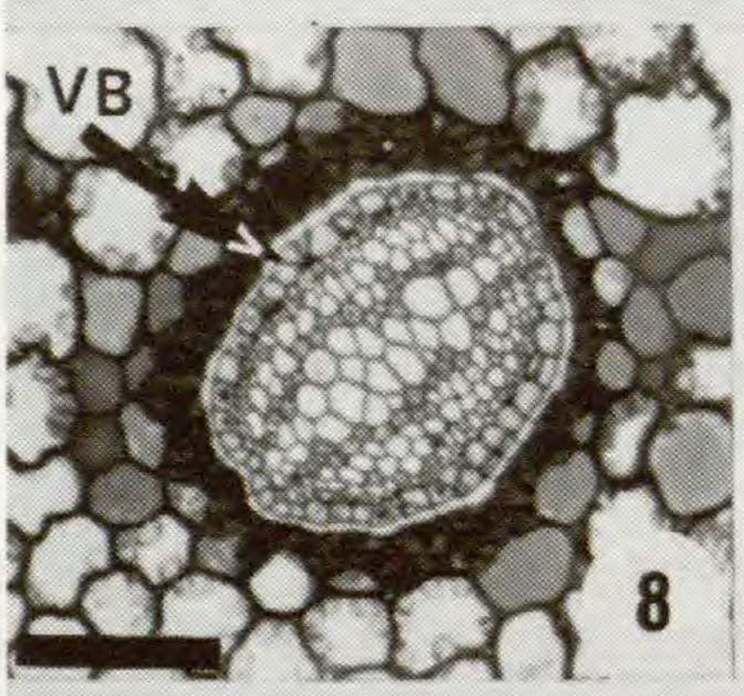


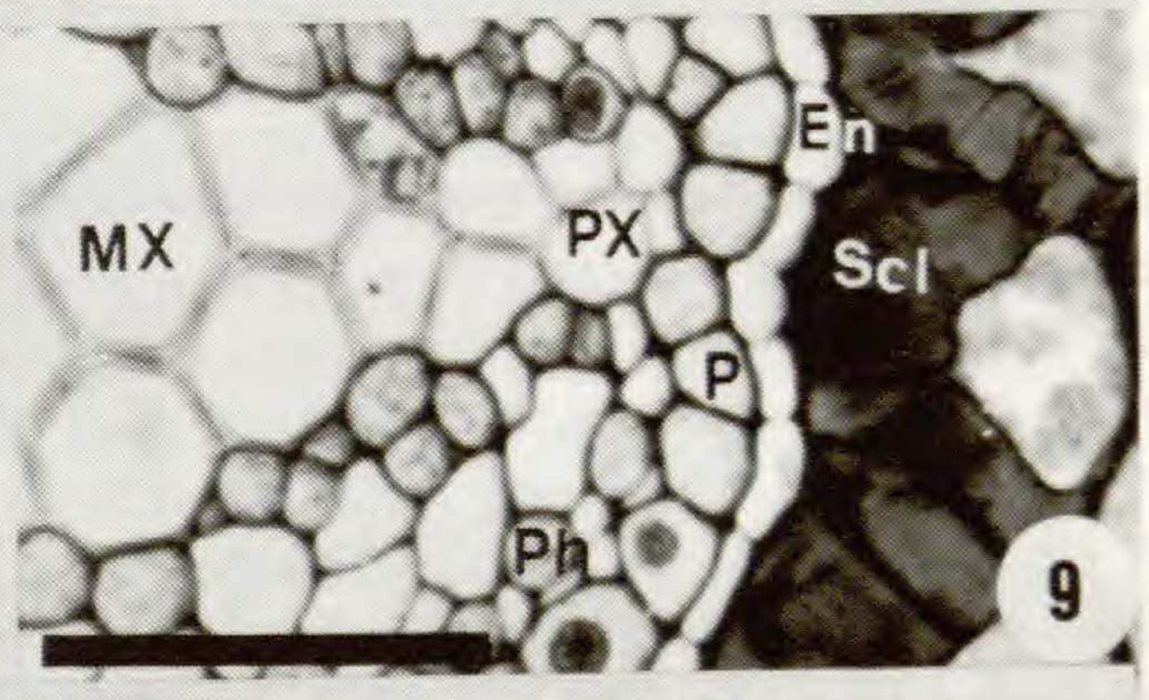
Figs. 1–3. *Microgramma squamulosa*. Fig. 1. General aspect of a plant without galls. Note the leaves and roots, respectively dorsal and ventral on the stems. Bar = 18 mm. Fig. 2. Note the characteristic vein pattern on the leaves. Bar = 10 mm. Fig. 3. General aspect of a plant with galls. Note the elongate fusiform shape of the stem gall. Where the gall occurs leaves and adventitious roots are generally lost. Observe the orifice by which the inductor leaves the gall (arrow). Bar = 18 mm.

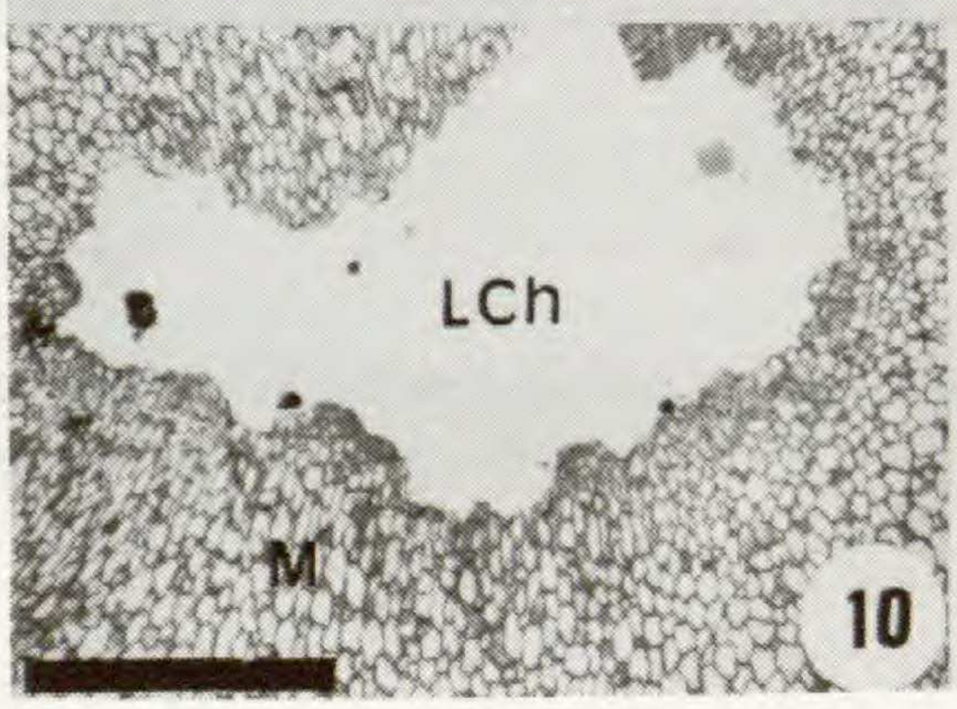


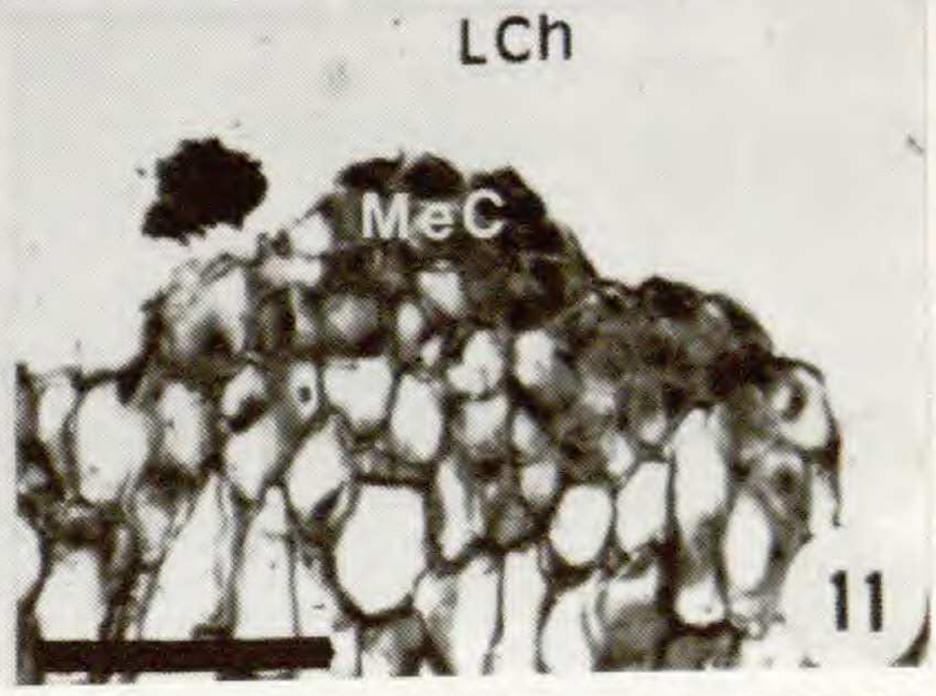












Figs. 4–11. *Microgramma squamulosa*. Figs. 4–9. Cross-section of a stem not affected. Figs. 9–10. Cross-section of young gall. Fig. 4. Note scales on the epidermis, cortex, stele and medulla (SEM). Bar = 300 µm. Fig. 5. Detail showing the scales on the epidermis. Bar = 500 µm. Fig. 6. Detail showing parenchymatic tissue of the cortex with nucleus and chloroplasts (SEM). Bar = 25 µm. Fig. 7. Note distribution of vascular bundles in the medulla. Bar = 1 mm. Fig. 8. General aspects of the vascular bundle. Bar = 22 µm. Fig. 9. Details of vascular bundle showing sclerenchyma, endodermis, pericycle, protoxylem, metaxylem, and phloem. Bar = 100 µm. Fig. 10. Note the larval chamber in the medullary region. Bar = 1 mm. Fig. 11. Detail of the meristematic cells of the larval chamber. Bar = 200 µm. C, cortex; Cl, chloroplast; E, epidermis; En, endodermis; LCh, larval chamber; M, medulla, MeC, meristematic cells; MX, metaxylem; N, nucleus; P, pericycle; Ph, phloem; PX, protoxylem; Sc, scale; Scl, sclerenchyma; St, stele; VB, vascular bundle.

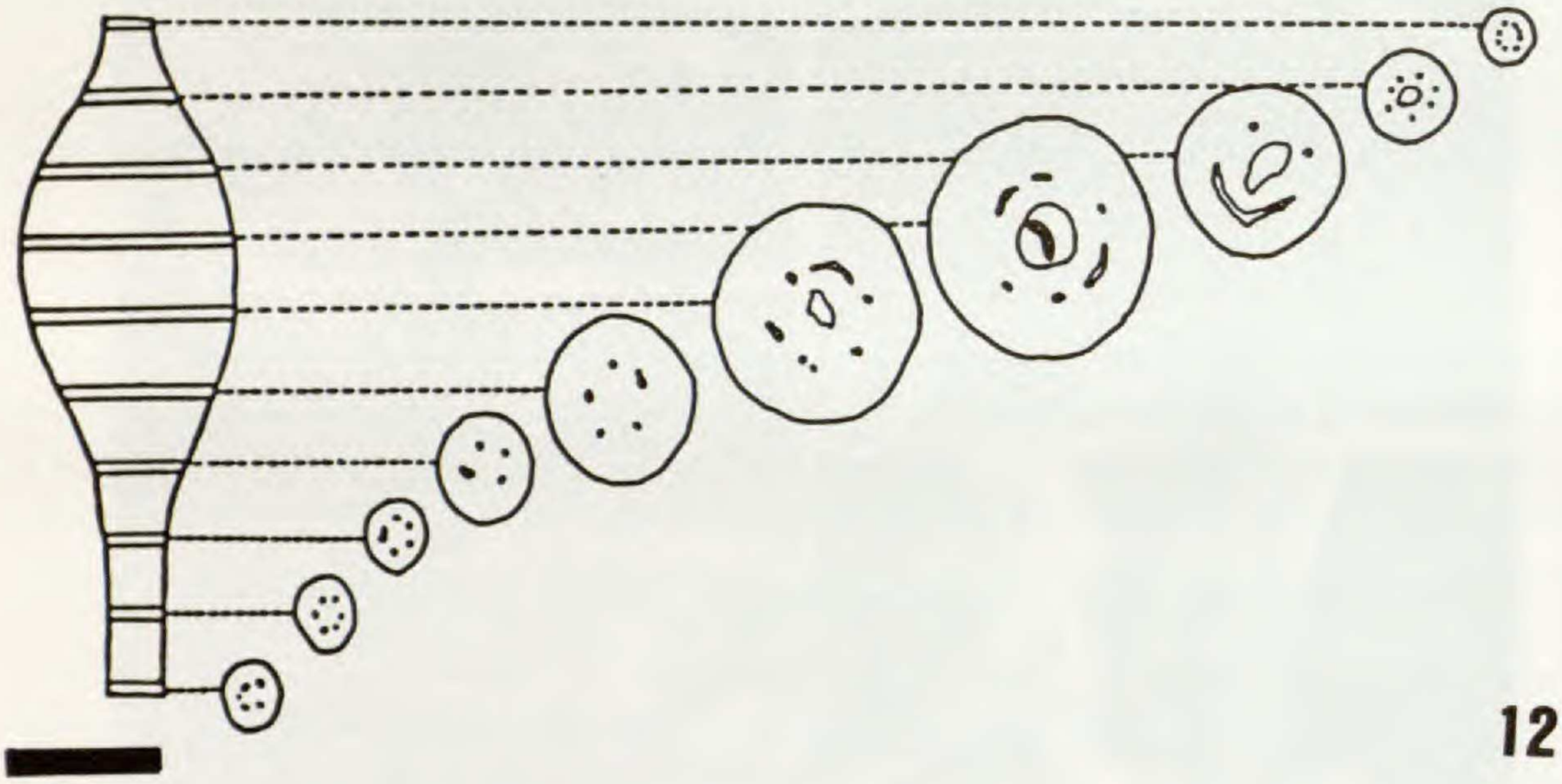


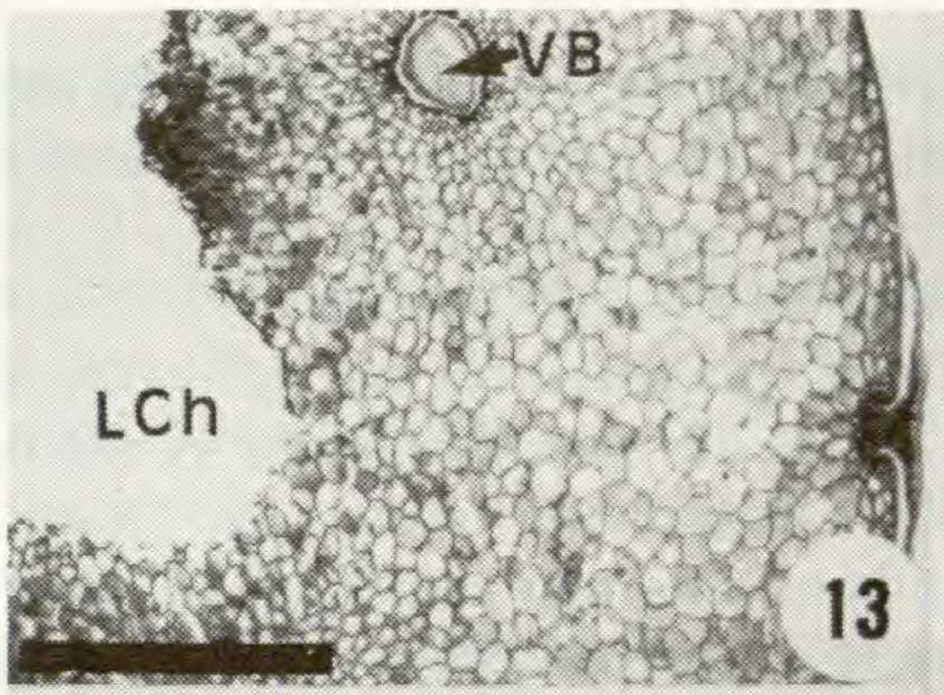
Fig. 12. *Microgramma squamulosa.* Diagram of cross-section series, showing bundle distribution in the gall. Bar = 10 mm.

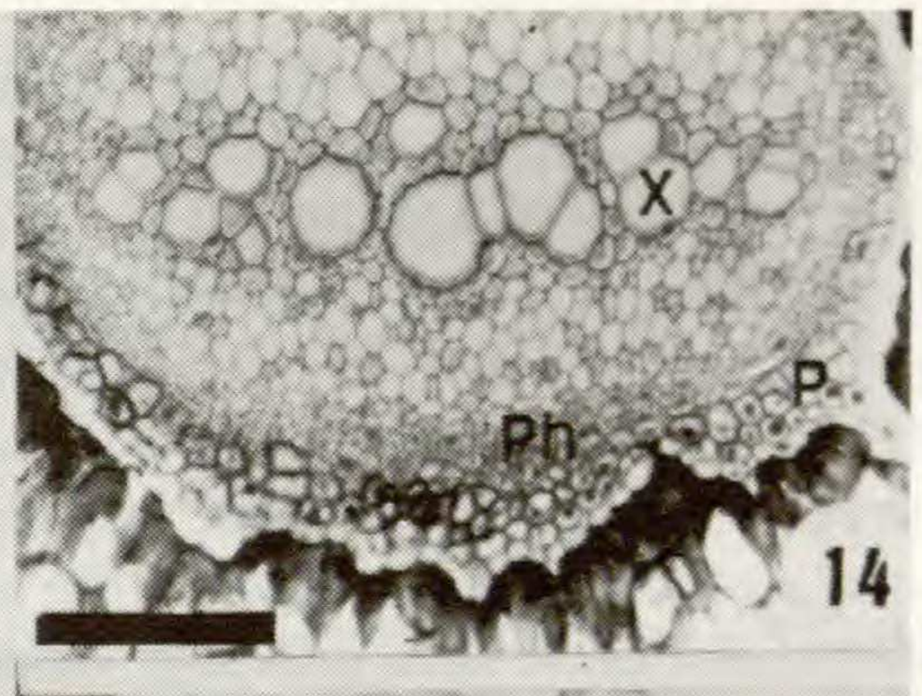
rectangular in shape, elongate in a tangential direction, and with thickening of the radial walls, characteristic of the presence of Casparian strips.

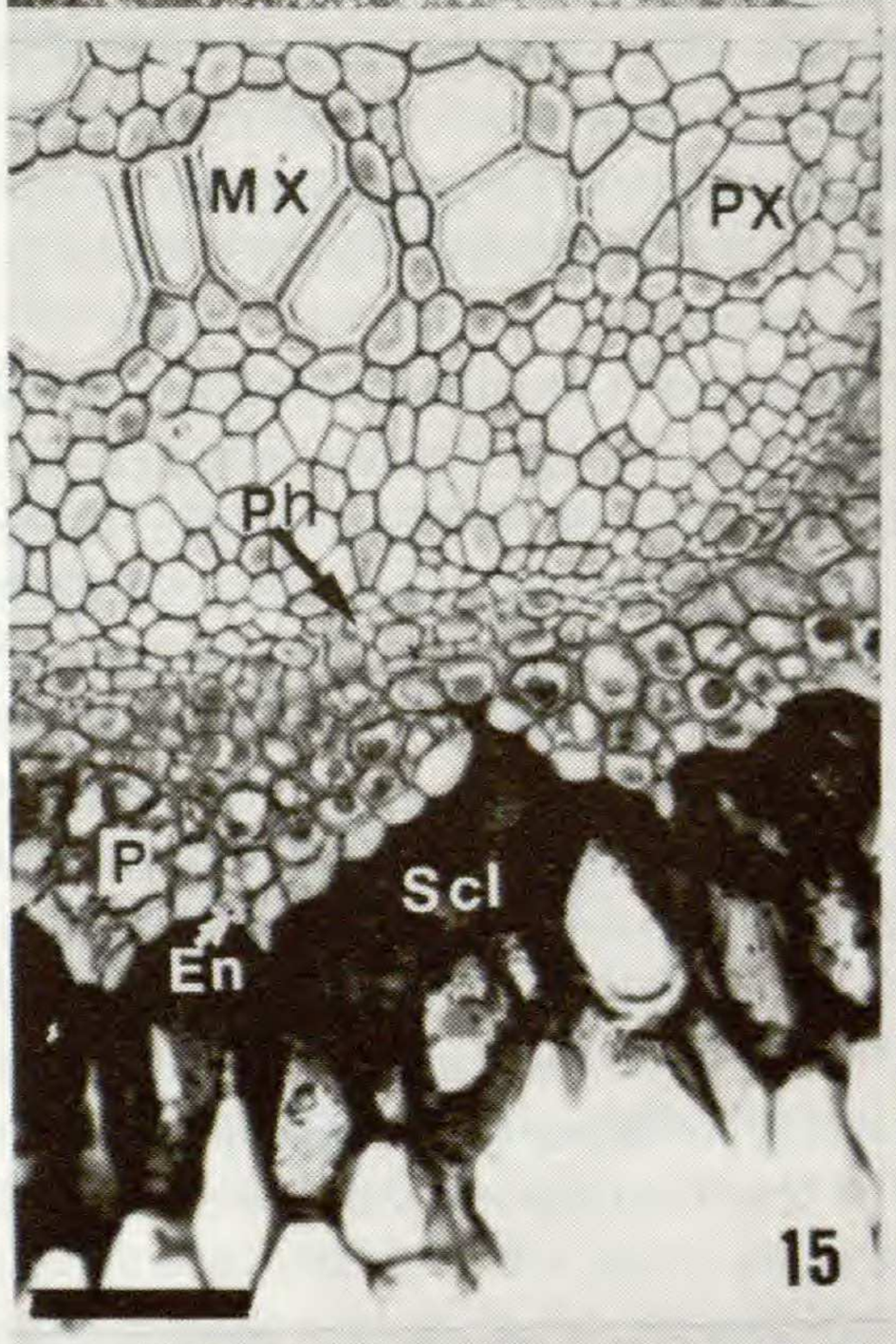
The entire vascular cylinder is concentric in organization; however, each meristele is formed by a bicolateral vascular bundle, with the phloem placed on both sides of the xylem (Fig. 8). The pericycle, situated between the phloem and endodermis, is generally made up of just one cell layer, quite distinct from both phloem and endodermis. Fig. 9 shows that, in association with the tracheal elements (tracheids) of the protoxylem and metaxylem, there occur the cells of the xylematic parenchyma, smaller and containing protoplasm. The conducting elements of the phloem (sieve cells) are quite simple in their pattern of differentiation: the protophloem remains at the periphery of each meristele, while the metaphloem differentiates centripetally. There is also the phloematic parenchyma. No significant difference has been observed between young and adult stems. Cross-sections of young galls show a relative small larval chamber formed in the medular parenchyma within the vascular cylinder (Fig. 10). The medullary cells which surround the larval chamber are meristematic in appearance – that is, the cells are smaller, contain dense cytoplasm, and have a conspicuous nucleus (Fig. 11). At this stage of gall development, no significant change is observed in the tissues of the vascular bundle.

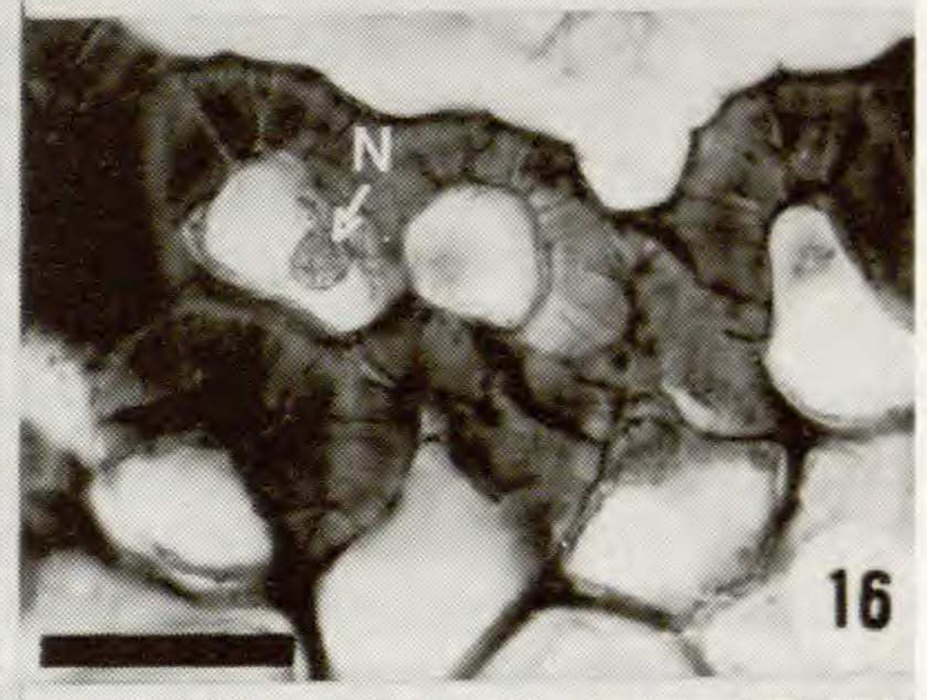
Cross-sections of stem segments at a more advanced stage of gall development (Fig. 12) show the occurrence of profound alteration in the distribution of the vascular bundles; these now display lateral expansions, forming bridges between neighboring meristeles. At this stage almost all the medullary region has been consumed by the larva, up to the immediate area of the vascular cylinder (Fig. 13). Profound alteration is also seen in the vascular bundle (Fig. 14 and 15) become larger, and their nucleus is also evident; the cytoplasm stains intensely with Safranin. Various layers of pericycle are formed as a result of cellular division.

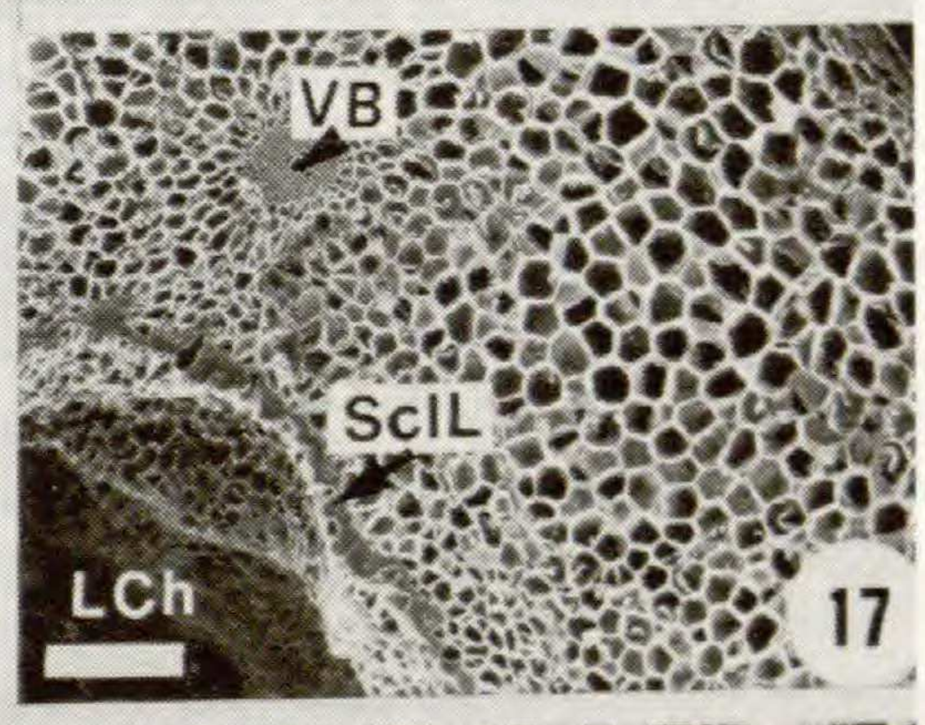
In cross-sections of the fully developed gall, the meristele has the same aspect (Fig. 16

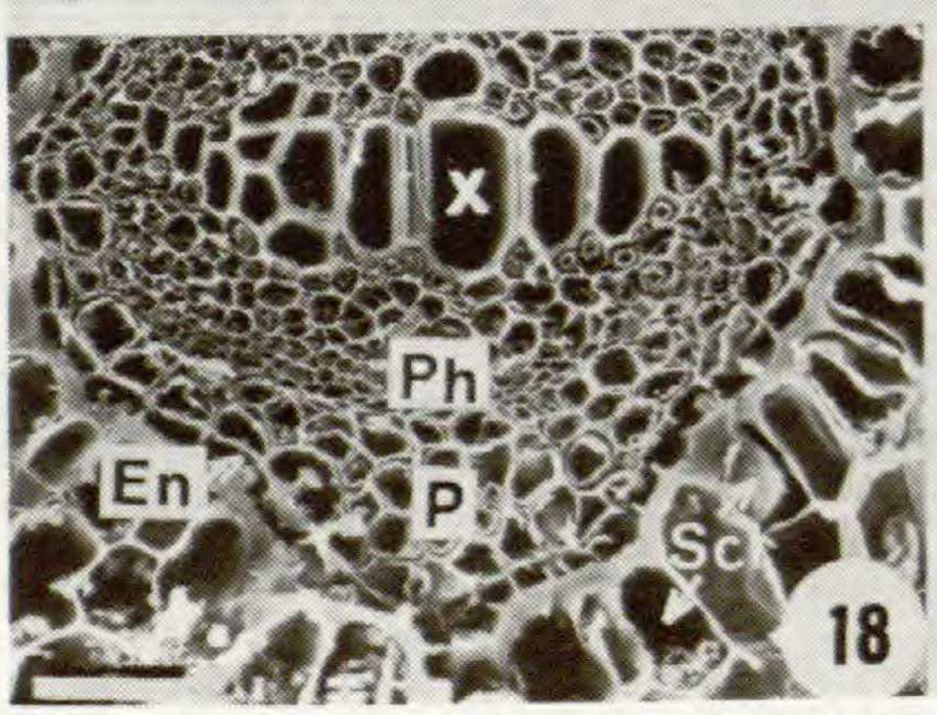


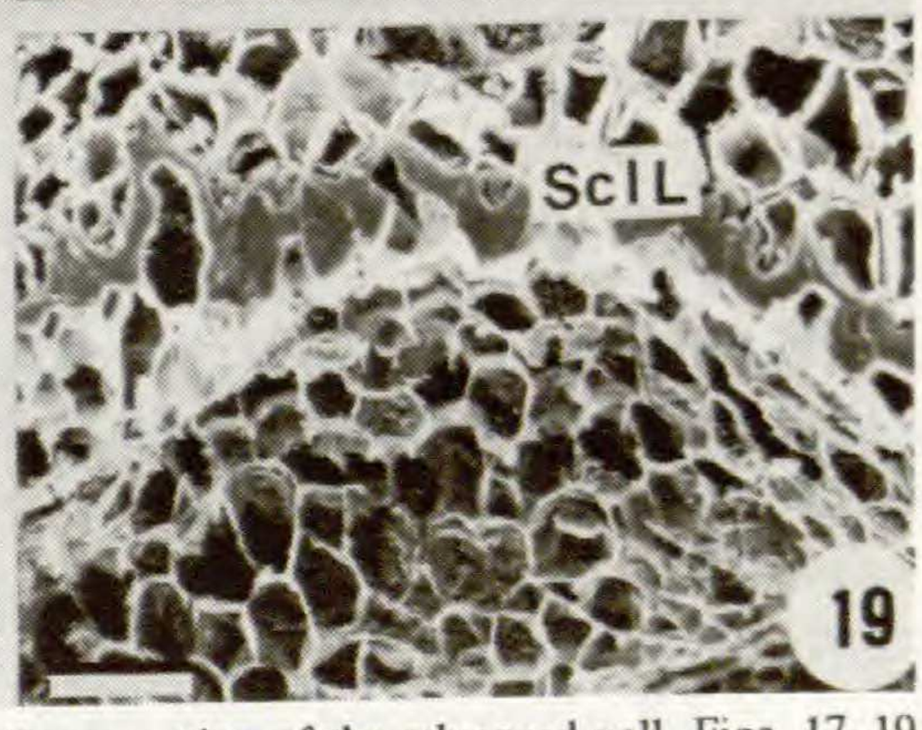












Figs. 13–19. *Microgramma squamulosa*. Figs. 13–16. Cross-section of the advanced gall. Figs. 17–19. Cross-section of fully developed gall. Fig. 13. Note the vascular bundle near the larval chamber. Bar = 1 mm. Fig. 14. Detail of the vascular bundle showing xylem, phloem and pericycle. Bar = 11 µm. Fig. 15. Details of part of the vascular bundle. Observe schlerenchyma, endodermis, cell layers of the pericycle, phloem and proto and metaxylem. Bar = 50 µm. Fig. 16. Note the nucleus of the living schlerenchyma cell. Bar = 25 µm. Fig. 17. Observe the larval chamber and the vascular bundle. Note the schlerenchyma-like tissue near the larval chamber (SEM). Bar = 300 µm. Fig. 18. Detail of the vascular bundle. Note cell layers of the pericycle, phloem and xylem (SEM). Bar = 30 µm. Fig. 19. Detail of the larval chamber. Note the layer of sclerenchyma-like cells around the larval chamber (SEM). Bar = 100 µm. En, endodermis; LCh, larval chamber; MX, metaxylem; N, nucleus; P, pericycle; Ph, phloem; PX, protoxylem; Scl, schlerenchyma; SclL, schlerenchyma-like cells; VB, vascular bundle; X, xylem.

and 17), but in the region of the medula, surrounding the larval chamber, the cells no longer show meristematic characteristics; there occurs instead a layer of cells with thickened walls, similar to the sclerenchyma cells (Figs. 16 and 18).

Discussion

The insect which induces the gall of *Microgramma squamulosa* belongs to the family Gelechiidae. According to Borror & Delong (1969) the microlepidoptera in this family have larvae with a variety of habits: there are leaf miners, leaf rollers, leaf cutters, gall inducers, and pests of stored grain. The authors state that gelechiids of the genus *Gnorimoschema* cause stem galls in a variety of plants, particularly in the Asteraceae. These galls are elongate, fusiform, with rather thin walls. The galls caused by *M. squamulosa* are also elongate, fusiform, but the walls are not thin. According to Borror & Delong (1969) the larva, before entering pupal phase inside the gall, prepares an aperture in the upper part, and leaves it partly closed. When the adult insect hatches, it can get out easily. This is also the case with the gall inducer studied here.

According to Ogura (1972) the family Polypodiaceae is distributed worldwide, and the fossil record dates back to the Palaeozoic. More than 1,000 species are known; most have creeping stems, covered with scales, and trichomes of a variety of forms. The leaves are dispersed along the stems. *M. squamulosa* lacks trichomes, but otherwise conforms to this general description. In the region where the gall is formed the stems usually lack leaves and aventitious roots. This effect of gall formation has not been studied here; it may be due to the involvement of hormones. It is interesting to note that, in this case, gall development does not involve developmental processes which are abnormal to the plant; the relevant cells and tissues are abnormal only in that they occur in unusual quantities and places. The same observation has been made for angiosperms by Mani (1964) and by Rohfritsch & Shorthouse (1982). In the case of *M. squamulosa*, the relevant modified structures and processes are the formation of nutritive tissue, the cellular proliferation in the region of the pericycle, and the presence of sclerified tissue around the larval chamber.

Structural and metabolic modifications in the cells close to the larval chamber have already been described by Rohfritsch (1971), Bronner (1977), Rohfritsch & Shorthouse (1982), Meyer & Maresquelle (1983) and Shorthouse (1986) for other types of gall. The modifications cause the formation of nutritive tissue. The cells of the nutritive tissue characteristically display dense cytoplasm, fragmented vacuoles, prominent nuclei and nucleoli, and a large number of organelles (Bonner, 1977; Rohfritsch & Shorthouse, 1982; Shorthouse, 1986). These characteristics are, in general terms, observed in the galls of *M. squamulosa*. According to Rohfritsch & Shorthouse (1982) the lepidopteran larvae which cause galls feed by mastigation, as is the case with the inducing larva of *M. squamulosa*, that actively consume the tissue mass which surrounds the larval chamber. Here the galls are caused by the action of the larva itself, and not, as in many other cases, including galls caused by Hymenoptera, by secretions deposited at the moment of oviposition.

The influence of gall formation on the vascular system of the host, even at some distance from the larval chamber, has been commented by Meyer (1969), Meyer & Maresquelle (1983) and by Arduin *et al.* (1991). The fact may cast some light on the cytological and histological alterations in the pericycle: the increase in the number of cells layers, and in the size of the cells and their nuclei, and the intense colour of the cytoplasm when stained with Safranin. This last feature may be associated with a possible

increase in the amount of phenolic substances in The cells, thus favouring impregnation with the stain. Phenolic substances have already been detected in the Polypodiaceae by Ogura (1972), but in other tissues. According to this author, the brown colour of the walls of the sclerenchymatic cells is probably due to impregnation with phlobaphgene, which would have the effect of preventing weakening of the tissue. According to Feeny (1970, 1976) and Tempel (1981), one of the functions of phenolic substances is to defend the plant against herbivory. The phenolic substances in the pericycle cells may also act in defense of the tissue.

The presence of mechanical tissues, made up of cells with thickened and lignified walls, is widespread in protoplasmic galls. The cells of gall schlerenchyma derive in general from parenchymatic cells (Mani, 1964). Thus the formation of sclerified tissue around the larval chamber in a fully-developed gall of *M. squamulosa* is not an exceptional case, and such cells are derived from the medullary parenchyma.

Although the idea that gall formation acts in defense of the plant has met with little acceptance, the observation of *M. squamulosa* seems to favour the hypothesis. The formation of a pericycle with various cell layers around the larval chamber may be a physical barrier, to prevent alterations to the conducting elements, fundamental for the survival of the plants. Taft & Bissing (1988) state that in oak stem galls, development causes a blockage of the conducting elements of the functional xylem, and where attack is very severe, branches or even the entire plant may die. Sclerenchyma development around the larval chamber in galls of *M. squamulosa* may thus impede the advance of the inducing larva up to the conducting tissue. But it should also be kept in mind that the formation of sclerified tissue may also serve to protect the inducing larva against predators (Cornell, 1983).

More profound study of galls in pteridophytes would be enlightening, since the relative anatomical simplicity of the group makes it possible to work on a perturbation of this nature using just a few variables; this is a model material for better understanding of the intricate interactions between insect and plant. According to Ogura (1972), the most differentiated tissue system in the pteriodphytes in general is the stele, which thus represents an important feature for anatomical investigation.

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Shorter Note

***Isoëtes reticulata* R. S. Hill 1987 (Alcheringa 12:158) is an illegitimate name.** — This name comes after *Isoëtes reticulata* C.B. Gena & T.N. Bhardwaja 1984. Three new species of genus Isoëtes L. from Rajasthan, India. (J. Bombay Natural History Society 81:165–168). The paper by Hill titled "Tertiary *Isoëtes* from Tasmania" (Alcheringa 12:157–162) is a very readable account about a Tasmanian fossil that is highly exciting to Isoetologists. There are four pictures of megaspores (Fig. 1, A–D) taken by SEM, showing striking reticulations of great clarity. These figures are compared with two from modern day species (Fig. 1, E–F). What is amazing is the fossil spores are from sediments containing leaf and root fragments that are not less than 22 million years old! Hill had sufficient quality material to assign it to the genus *Isoëtes* rather than the fossil genus *Isoëtites.* I am pleased to name this interesting fossil *Isoëtes* from Tasmania in honor of Dr. R.S. Hill.

***Isoëtes hillii* D. M. Britton, nom. nov. for *I. reticulata* Hill (1987), non Gena & Bhardwaja (1984).** — Donald M. Britton, Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.